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(54) **Title:** SILVER DIHYDROGEN CITRATE COMPOSITIONS COMPRISING A SECOND ANTIMICROBIAL AGENT

(57) **Abstract:** Composition comprising silver dihydrogen citrate in combination with a second antimicrobial compound are provided. The second antimicrobial compound may be quaternary ammonia, an oxidizer or a halogen species, such as chlorine, bromine or iodine. Methods of using the antimicrobial composition provide superior antimicrobial effects.

SILVER DIHYDROGEN CITRATE COMPOSITIONS COMPRISING A SECOND ANTIMICROBIAL AGENT

FIELD OF THE INVENTION

The invention relates to antimicrobial compositions and their methods of use. In particular, the invention relates to silver dihydrogen citrate compositions comprising quaternary amines, an oxidizing agent or a halogen compound, and methods of using the compositions to confer an antimicrobial effect on various substrates.

BACKGROUND OF THE INVENTION

A large number of antimicrobial compounds are known. Compounds that kill or prevent the spread of microbes have been used for a variety of purposes and in numerous products to prolong product shelf-life, and to confer antimicrobial benefits to end-users, their possessions and their environments. Some antimicrobial compounds that may be mentioned include quaternary ammonium compounds, oxidizing agents and halogen compounds.

Quaternary ammonium compounds are cationic surfactants that have been shown to have antibacterial properties. In addition to antibacterial properties, some quaternary ammonium compounds also possess deodorant, wetting, detergent, keratolytic and emulsifying activities. Benzalkonium chlorides are mixtures of alkylbenzyldimethyl ammonium chlorides, and are widely used as antiseptics in clinical settings. Benzalkonium chlorides are also used as preservatives, for example in ophthalmic solutions, due to their activity against various bacteria, including Gram-positive and some Gram-negative bacteria, as well as some viruses and fungi. However, benzalkonium chlorides are not effective against some Gram-negative bacteria, such as *Pseudomonas*. While such compounds are beneficial at relatively low concentrations, at higher concentrations they can be irritating or even toxic. Also, quaternary compounds tend to dissipate in the environment quickly, and thus tend not to provide extended antimicrobial effects when applied to solid surfaces and other substrates.

Oxidizing agents, such as hydrogen peroxide, have been used as disinfectants. Hydrogen peroxide is known as a disinfectant for disinfecting skin and wounds and for

disinfecting water supplies. Potassium permanganate ($KMnO_4$) is an oxidizing agent that has been used as a bactericide, fungicide and algicide in cooling towers, evaporative condensers, air wash systems, cooling fountains, and in human drinking water. Potassium permanganate has also been used to control external infections of fish in aquariums and ornamental ponds. Monopersulfates, such as potassium monopersulfate, are used as disinfectants and as shocking agents for the occasional disinfection of swimming pools and spas. In use as shocking agents, monopersulfates oxidize and break down chloramines, thereby increasing the effective concentration of free chlorine or in chlorine-treated water. Other oxidizing agents that may be mentioned are benzoyl peroxide and sodium perborate, which have also been used as antimicrobial compounds. While oxidizing agents are very effective disinfectants, they tend to degrade quickly in the environment, and thus are not suitable for providing antimicrobial effects over an extended period of time.

Halogen compounds have also been used as disinfectants. Although they may be thought of as oxidants, halogen compounds are categorized separately herein, in view of their special characteristics. The most commonly used antimicrobial halogen compounds are chlorine and iodine. Bromine is also used in some situations, as where a person who may come into contact with the antimicrobial is known to have a sensitivity to chlorine.

Iodine is one of the oldest known disinfectants, and is used for wound treatment, skin disinfection and water sanitization. Iodine may be used as an iodine tincture or as an iodophor. Iodine tincture, USP, is a 2% solution of free iodine with 2.4% sodium iodide in 50% ethyl alcohol or isopropyl alcohol. Tinctures can be applied directly to skin for disinfection and wound treatment, or can be diluted in water for disinfection.

An iodophor is a composition including iodine, detergents, wetting agents, solubilizers, and other carriers. Iodophors often contain as much as 30% iodine by weight, of which about 70-80% may be released as available iodine upon dilution. These compounds are safe with low toxicity and almost no odor. While not as irritating as tinctures, when used on skin iodophors can act as desiccants and can result in dermatitis.

Chlorine may be prepared *in situ* by electrolysis of an electrolyte solution containing chloride ions. Chlorine made in this manner is short-lived, but is a highly effective water

disinfectant, for instance in swimming pools, spas and drinking water systems. Chlorine may also be prepared as liquid bleach, that is as sodium hypochlorite. Chlorine may also be prepared as a solid hypochlorite, which is often formed into cakes or tablets that may be directly placed within water containers to produce the desired disinfectant effect.

Bromine is less-widely used as a skin disinfectant and wound treatment than is iodine. Bromine has also been used as a sanitizing agent in cooling towers, swimming pools and spas. When dissolved in water, liquid bromine forms disinfectant hypobromous acid. Disinfectant bromine is often sold to the public as a concentrated liquid in water.

All the halogens are potentially irritating, especially to the eyes, mucus membranes and open wounds. Additionally, the halogens are also potentially toxic when ingested. Thus, it is generally desirable to use the halogens for disinfection at their lowest effective concentration.

Antimicrobial metal ion solutions are commonly used as disinfectants. Silver solutions have been used as disinfectants in cooling towers, swimming pools, hot water systems in hospitals, portable water systems and spa pools. Additionally, silver ion solutions have been prepared for the treatment of wounds, however the silver ions used in the proposed methods are unstable and must be generated near the wound in order to deliver a therapeutic dose to the wound site.

Silver salts, such as silver citrate salts, have also been proposed as antimicrobial dusting agents. However, these dusting agents must be kept dry and are generally not convenient for imparting preservative value to consumer products or for delivering antimicrobial effects to an end user or to the environment of the end user. Colloidal silver has found a variety of uses, including: as a wood preservative; as a disinfectant of food and beverage containers and industrial processing equipment; as a bactericide in paints; as a biocide in synthetic polymer films; and as a sterilizing agent in bandages.

Aqueous solutions of silver dihydrogen citrate and citric acid have been disclosed in US Patent No. 6,197,814 (incorporated herein in its entirety) as disinfectants in a variety of settings. These water solutions of silver dihydrogen citrate and citric acid are made by passing an electrical current through a pair of silver electrodes that are immersed in a water

solution of citric acid. These silver dihydrogen citrate solutions are effective against a wide-variety of microbes, including bacteria, viruses and fungi, and are non-toxic in the human environment at concentrations effective to combat microbial infestation.

Despite the antimicrobial efficacy of various known antimicrobial compounds, there remains a need for antimicrobial compositions that are effective at lower concentrations of antimicrobial agent. There is also a need for antimicrobial compositions that provide the advantages of quaternary amines, oxidizing agents and halogen compounds, but in addition possess extended antimicrobial effect on solid surfaces and other substrates. The present invention satisfies these needs and provides related advantages as well.

SUMMARY OF THE INVENTION

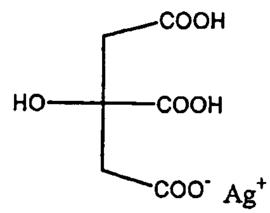
The present invention provides antimicrobial compositions of matter. The compositions comprise silver dihydrogen citrate, citric acid and a second antimicrobial agent. The second antimicrobial agent is selected from the group consisting of quaternary ammonium compounds, oxidizing agents and a halogen compounds.

The invention also provides a method of conferring an antimicrobial effect on a substrate. The method includes applying an antimicrobial composition to a substrate. The antimicrobial composition comprises silver dihydrogen citrate, citric acid and a member of the group consisting of quaternary ammonium compounds, oxidizing agents and halogen compounds.

DETAILED DESCRIPTION OF THE INVENTION

The invention described herein provides a composition having activity against microbes, such as bacteria, viruses and fungi. The compositions comprise silver dihydrogen citrate, citric acid and a member of the group consisting of quaternary ammonium compounds, oxidizing agents and halogen compounds. The invention further provides antimicrobial methods of using the aforementioned compositions.

As used herein, the term "silver dihydrogen citrate" refers to molecule having the chemical formula $AgC_6H_7O_7$. The chemical structure is represented by formula I:



(I)

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and its positional isomers, such as where the Ag^+ ion is on the 3-carboxylic acid group rather than the 1-carboxylic acid group as depicted. The person skilled in the art will recognize that the compound of formula I is a salt. Thus, silver dihydrogen citrate will exist in a dissociated state in solution.

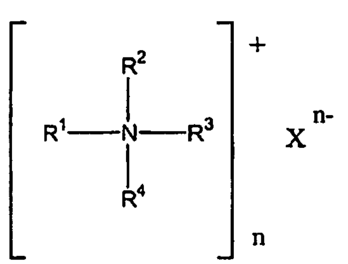
The invention provides compositions comprising silver dihydrogen citrate as a first antimicrobial agent, citric acid and a second antimicrobial agent. The second antimicrobial agent is advantageously selected from the group consisting of quaternary ammonium compounds, oxidizing agents and halogen compounds. In combination, the silver dihydrogen citrate, citric acid and second antimicrobial agent give rise to improved antimicrobial effects, due to the combined antimicrobial activity of silver ion (Ag^+) and the second antimicrobial agent. In some embodiments, the compositions provide immediate antimicrobial effects due to the combination of silver dihydrogen citrate and the second antimicrobial agent, as well as extended antimicrobial effects due to the persistent antimicrobial action of silver dihydrogen citrate on the treated substrate, notwithstanding the degradation of the second antimicrobial agent. In such embodiments, the silver dihydrogen citrate, upon drying, forms a crystalline residue that imparts long-lasting antimicrobial effects. For example a composition of silver dihydrogen citrate, citric acid and a quaternary ammonium compound provides immediate antimicrobial effects against a broad spectrum of microbes, including *Pseudomonas*, as well as extended antimicrobial effects when applied to a substrate, such as a surface or an article. As another example, a composition of silver dihydrogen citrate, citric acid and an oxidizing agent provides immediate antimicrobial activity due to the combination of silver dihydrogen citrate and oxidizing agent, as well as long-lasting antimicrobial benefits when applied to a substrate.

In other embodiments, the combination of silver dihydrogen citrate and the second antimicrobial agent give rise to a synergistic effect. The term "synergistic" as defined herein means that the combined effect of two or more agents is greater than the effect achievable with the two agents taken separately. In general, this means that, for a particular organism, when the following formula is satisfied, the combination of two active agents A and B is synergistic:

$$EC_{50}(A+B) < EC_{50}(A)+EC_{50}(B)$$

wherein EC_{50} means the concentration effective to bring about a 50% reduction in a measure of organism activity. A and B represent the two antibacterial agents, $EC_{50}(A+B)$ is the EC_{50} for the combination of A and B, whereas $EC_{50}(A)$ is the EC_{50} for A alone and $EC_{50}(B)$ is the EC_{50} for B alone. Organism activity can be either organism proliferation or organism vitality. For example, where the antimicrobial effect that is desired is a bacteriostatic effect, EC_{50} is a measure of the concentration at which the agent will reduce by 50% the organism's rate of cell division. Where the antimicrobial effect is bacteriocidal, EC_{50} is the concentration at which the agent will reduce the number of live organisms by 50%. For example, a composition comprising silver dihydrogen citrate, citric acid and a halogen compound will require less than half the concentration of each of silver dihydrogen citrate and halogen compound as is necessary when each is used separately when used as an antibacterial, antifungal or antiviral.

The invention provides silver dihydrogen citrate compositions comprising quaternary ammonium compounds. Quaternary ammonium compounds can be represented by formula II or III: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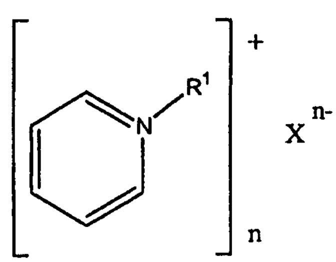
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wherein each of R^1, R^2, R^3 and R^4 is independently an organic radical, n is an integer and X^{n-} is an anion (counter-ion) having a negative charge of n.

Appropriate organic radicals in formulas II and III above include alkyl, alkenyl, alkynyl, alkyl having at least one substituent, alkenyl having at least one substituent, and alkynyl having at least one substituent.

In formulas II and III, alkyl means C_1-C_{30}, optionally branched, fully saturated hydrocarbyl. Alkenyl means C_2-C_{30}, optionally branched, partially or fully unsaturated hydrocarbyl, wherein each unsaturation is a double carbon-carbon bond. Alkynyl means C_2-C_{30}, optionally branched, fully or partially unsaturated hydrocarbyl, wherein at least one unsaturation is a triple carbon-carbon bond. Suitable alkyl groups include methyl, ethyl, isopropyl, n-propyl, n-butyl, t-butyl, s-butyl, and optionally branched pentyl, hexyl, heptyl, octyl, nonyl, decyl, undecyl, dodecyl, triskaidecyl, octadecyl, eicosyl (C_{20}), etc. Suitable alkenyl groups include: ethenyl, 1-propenyl, 2-propenyl, 3-propenyl, 1-buten-4-yl, 2-buten-4-yl, and optionally branched pentenyl, hexenyl, heptenyl, octyenl, nonenyl, decenyl, undecenyl, dodecenyl, triskadecenyl, octadecenyl, 5,8,11,14-eicosatetraenyl, 9,12-octadecadienyl, 9,12,15-octadecatrienyl, etc. Suitable alkynyl groups include: ethynyl, 1-propynyl, 3-propynyl, 2-butyn-1-yl, etc.

Substituents for the foregoing alkyl, alkenyl and alkynyl groups include: cycloalkyl (saturated or partially unsaturated), aryl, heterocyclyl (saturated or partially unsaturated), heteroaryl, fluoro, chloro, bromo, iodo, hydroxyl, sulfhydryl, nitro, etc. Cycloalkyl includes saturated cycloalkyl, such as cyclopropyl, cyclobutyl, cyclopentyl, cylcohexyl, cylcopentyl, cyclooctyl, etc. Aryl includes: phenyl, naphthyl, anthracenyl, etc. Heterocyclyl includes pyrrolidinyl, piperidinyl, piperazinyl, morpholino, thiomorpholino, etc. Heteroaryl includes pyridyl, thiophenyl, furanyl, thiazolyl, pyrimidinyl, etc.

The invention provides compositions comprising silver dihydrogen citrate and citric acid in combination with particular quaternary ammonium compounds of formulas II and II, wherein each of R^1, R^2, R^3 and R^4 is methyl, ethyl, isopropyl, n-propyl, n-butyl, t-butyl, s-butyl, C_4-C_{20} alkyl, or one of the following having a substituent: methyl, ethyl, isopropyl, n-propyl, n-butyl, t-butyl, s-butyl, or C_4-C_{20} alkyl, wherein the substituent is aryl, in particular

phenyl. Particular values of R^1, R^2, R^3 and R^4 that may be mentioned are methyl, ethyl, isopropyl, n-propyl, dodecyl, octadecyl, eicosyl, benzyl, phenylethyl, etc.

The invention provides particular compositions comprising silver dihydrogen citrate, citric acid and a quaternary ammonium compound of formula II or III, wherein the counter-ion is an anion having a charge of -1, -2 or -3. Suitable counter-ions include: chloride, bromide, iodide, nitrite, nitrate, bisulfite, sulfite, bisulfate, sulfate, phosphate, hydrogen phosphate, dihydrogen phosphate, hydroxide, etc.

The invention provides particular compositions comprising silver dihydrogen citrate, citric acid and particular quaternary ammonium compounds, such as: tetrabutyl ammonium bromide, tetrabutyl ammonium hydrogen sulfate, tetrabutyl ammonium fluoride, tetrabutyl ammonium chloride, tetraethyl ammonium bromide, tetraethyl ammonium chloride, tetraethyl ammonium iodide, tetrapropyl ammonium bromide, tetrapropyl ammonium iodide, tetramethyl ammonium chloride, tetramethyl ammonium bromide, tetramethyl ammonium iodide, tetramethyl ammonium hydroxide, tetraoctyl ammonium bromide, tetraoctyl ammonium chloride, tetraoctyl ammonium iodide, tetraoctyl ammonium hydroxide, benzyl triethyl ammonium chloride, benzyl tributyl ammonium chloride, benzyl trimethyl ammonium chloride, benzyl trimethyl ammonium dichloro iodide, benzyl trimethyl ammonium bromide, butyl triethyl ammonium bromide, methyl trioctyl ammonium chloride, methyl tricapryl ammonium chloride, methyl tributyl ammonium chloride, myristyl trimethyl ammonium bromide, cetrimide, cetyl trimethyl ammonium bromide, lauryl trimethyl ammonium chloride, phenyl trimethyl ammonium chloride, benzalkonium chloride, tetrabutyl ammonium perchlorate, tetrabutyl ammonium p-toluene sulfonate, tetraethyl ammonium p-toluene sulfonate, cetyl trimethyl ammonium p-toluene sulfonate, tetraethyl ammonium tosylate, tetrabutyl ammonium tosylate, cetyl trimethyl ammonium tosylate, cetyl pyridinium chloride, cetyl pyridinium bromide, lauryl pyridinium chloride and lauryl pyridinium bromide.

The invention provides compositions comprising silver dihydrogen citrate, citric acid and a quaternary ammonium compound in various concentrations, depending upon the intended use of the compositions. For example, the invention provides compositions

comprising at least about 0.5 ppm silver dihydrogen citrate and at least about 50 ppm quaternary ammonium compound.

The invention also provides compositions comprising silver dihydrogen citrate, citric acid, quaternary ammonium compound and a dispersing agent. Suitable dispersing agents are generally surfactants, such as sodium dodecyl sulfate or Octoxynol (polyethylene glycol octadecyl ether). Such compositions comprise greater than about 0.01% (wt./wt.) dispersing agent.

The invention provides compositions comprising combinations of silver dihydrogen citrate, citric acid and one or more oxidizing agents. Oxidizing agents that may be mentioned are peroxides, such as hydrogen peroxide and benzoyl peroxide; permanganates, such as potassium permanganate, persulfates, such as potassium monopersulfate.

The invention provides compositions comprising silver dihydrogen citrate, citric acid and an oxidizing agent in various concentrations, depending upon the intended use of the compositions. For example, the invention provides compositions comprising at least about 0.5 ppm silver dihydrogen citrate and at least about 50 ppm oxidizing agent.

The invention also provides compositions comprising silver dihydrogen citrate, citric acid, oxidizing agent and a dispersing agent. Suitable dispersing agents are generally surfactants, such as sodium dodecyl sulfate or Octoxynol. Such compositions comprise greater than about 0.01% (wt./wt.) dispersing agent.

The invention provides compositions comprising combinations of silver dihydrogen citrate, citric acid and one or more halogen compounds. Halogen compounds that may be mentioned are iodine (I_2), bromine (Br_2), chlorine (Cl_2), sodium hypochlorite, calcium hypochlorite, etc. When iodine is used as an ingredient, it may be added as a tincture or an iodophor. When bromine is used as an ingredient, it may be added as liquid bromine or as a dilute solution. When chlorine is used as an ingredient, it may be bubbled into the solution as Cl_2 gas, or it may be added as a liquid or solid hypochlorite compound.

The invention provides compositions comprising silver dihydrogen citrate, citric acid and halogen compound in various concentrations, depending upon the intended use of the

compositions. For example, the invention provides compositions comprising at least about 0.1 ppm silver dihydrogen citrate and at least about 2 ppm halogen compound.

The invention also provides compositions comprising silver dihydrogen citrate, citric acid, halogen compound and a dispersing agent. Suitable dispersing agents are generally surfactants, such as sodium dodecyl sulfate or Octoxynol. Such compositions comprise greater than about 0.01% dispersing agent.

Generally, silver dihydrogen citrate of formula I can be made by immersing silver electrodes in an aqueous electrolyte solution that contains citric acid. An electrolytic potential is then applied to the electrodes, whereby silver ion is generated in the solution. When combined in this way, silver ions and citric acid form silver dihydrogen citrate, which is stable in aqueous citric acid solution. In some embodiments of the invention, the electrolyte contains greater than about 5%, particularly greater than about 10% citric acid (% wt/volume), and more particularly 20% or greater. The silver dihydrogen citrate is then be combined with other ingredients as further described herein.

Compositions of silver dihydrogen citrate, citric acid and at least one member of the group consisting of a quaternary ammonium compound, an oxidizing agent (oxidizer) and a halogen compound may be made by combining a solution of silver dihydrogen citrate with one or more appropriate ingredients selected from quaternary ammonium compounds, oxidizing agents, halogen compounds and combinations thereof. In some embodiments, the silver dihydrogen citrate solution as described above is diluted to an appropriate concentration to create a working solution, to which the ingredient is added in an appropriate amount, with mixing. Optionally further antimicrobial ingredients, dispersing agents or combinations thereof may be added to the working solution. When all the desired ingredients are added and fully integrated into the solution, the solution is then further diluted with water to form the final composition. The person skilled in the art will recognize that the order of addition of silver dihydrogen citrate and other ingredients may be altered as desired without affecting the efficacy of the solution.

Silver dihydrogen citrate has been shown to be antimicrobial against a variety of organisms, including bacteria, fungi and viruses. Particular microbes against which efficacy

has been demonstrated include *Pseudomonas aeruginosa* (especially ATCC 15442), *Salmonella choleraesuis* (especially ATCC 10708), *Staphylococcus aureus* (especially ATCC 65328 and ATCC 700698), *E. coli* (especially 0157:H7, ATCC 43888 and ATCC 11229), *Listeria monocytogenes* (especially ATCC 11543 and 19111), *Enterococcus faecium* (especially ATCC 6569 and ATCC 700221), Human immunodeficiency virus 1 (HIV 1), Herpes simplex virus type 1 (HSV 1), Poliovirus type 2, Influenza A, Rhinovirus, *Propionibacterium acnes* (especially ATCC 6921), *Trichophyton mentagrophytes* (especially ATCC 9533). Thus, the invention provides compositions having activity against a broad spectrum of microbes, including bacterial species, such as species of the genera: *Pseudomonas, Salmonella, Staphylococcus, Escherichia, Listeria, Enterococcus and Propionibacterium*. The invention also provides compositions having broad-spectrum activity against various viruses. The invention further provides compositions having activity against dermatophytes, such as species of the genus *Trichophyton*.

Quaternary ammonium compounds have been shown to have antimicrobial effects against a broad spectrum of microbes, including Gram-positive and Gram-negative bacteria, as well as some viruses and fungi. In particular, benzalkonium chloride has been found effective against the viruses mumps, rotavirus, rubella, measles, and HIV.

Oxidizing agents have been shown to have a broad spectrum antimicrobial effects. For example, 3% hydrogen peroxide solutions in water have been used as topical antiseptics. Hydrogen peroxide (3%) has also been used in a combination treatment with 5 % acetic acid (vinegar) solution as a surface treatment to kill *Salmonella* and other bacterial species. Potassium permanganate ($KMnO_4$) is an oxidizing agent that has been used as a bactericide, fungicide and algicide in cooling towers, evaporative condensers, air wash systems, cooling fountains, and in human drinking water. Potassium permanganate has also been used to control external infections of fish in aquariums and ornamental ponds. Monopersulfates, such as potassium monopersulfate, are used as oxidizing agents in the shocking of swimming pools and spas. Other oxidizing agents that may be mentioned are benzoyl peroxide and sodium perborate. Benzoyl peroxide, 5% or 10% solution has been used as an acne treatment.

Halogen compounds also have been shown to have broad-spectrum antimicrobial effects. Chlorine is considered a front-line disinfectant for use in the treatment of water, such as in water supplies, cooling towers, swimming pools and spas. It is often used in concentrations of approximately 1,000 ppm although in swimming pools and spas its concentration is generally between 3 and 5 ppm of total chlorine. Bromine has been used as an antiseptic and disinfectant. Bromine is effective as an antiseptic at about 1,000 ppm concentration, and as a water treatment at about 4 to 6 ppm. Iodine is used both as a disinfectant and as an antiseptic. It is generally used as an antiseptic at a concentration of about 1 to 2 %. Povidone iodine has been shown to have activity against a broad spectrum of bacteria, including *Prbteus, Staphylococcus, Pseudomonas, Streptococcus, Escherichia, Salmonells, Candida, Serratia, Spores-Baccillus; Clostridium, Trichomomonas, Enterobacter, Klebsiella, Clostridium, Shigella, Corynebacterium, Diplococcus, Mycobacterium, Bacillus, Sarcina, Trichophyton, Aspergillus, Mima, Herella, Edwardsiella, Citrobacter, Providencia, Acienetobacter, Epidermophyton, Microsporum, Pencillium and Nocardia*. In addition to activity against a broad spectrum of bacteria, povidone iodine (an iodophor) has been found effective in immobilizing adenovirus, mumps virus, rotavirus, poliovirus, coxsackievirus, rhionviurs, herpes simplex virus, rubella, measles, influenza and human immunodeficiency virus (HIV). (See R. Kawana et al., Dermatology 195, Supp. 2, 29-35 (1997).

The invention provides methods of using antimicrobial compositions comprising silver dihydrogen citrate, citric acid and a second antimicrobial agent selected from quaternary ammonium compounds, oxidizing agents and halogens. The invention also provides methods of treating various substrates with the antimicrobial compositions to achieve an antimicrobial effect. The antimicrobial effect may be antibacterial, antiviral and/or antifungal. The antibacterial effect may be bacteriocidal or bacteriostatic. Antiviral effects include immobilization and disruption of virions. Antifungal effects include fungicidal and fungistatic effects. The method comprises applying to a substrate an amount of the treatment composition effective to bring about the desired antimicrobial effect.

The term "substrate" is generically used herein to mean any surface, article or environment that is in need of antimicrobial treatment. The invention provides treating

various surfaces, including countertops, floors, glass surfaces, metal surfaces (such as stainless steel, chrome and copper surfaces), tile surfaces, concrete surfaces, vinyl flooring and painted surfaces. The term "surface" is used herein to connote any surface, including interior and exterior surfaces of various objects, including interiors of containers (such as boilers, water tanks, swimming pools, etc.), interiors of pipes, exteriors of household fixtures and appliances, countertops, glass windows and doors. The term "surface" is used herein to distinguish over a whole article. The antimicrobial solutions are applied to the surface in a conventional manner, such as by pouring, spraying or swabbing the solution onto the surface. The solutions are conveniently wiped or rinsed off the surface; however in some preferred embodiments, they are left on the surface to dry, thereby providing a long-lasting antimicrobial residue or film on the surface.

The invention provides methods of treating other substrates, including various articles, such as fabrics, metal articles, plastic articles, natural products and other articles that are often treated with aqueous cleaning solutions. For example, the invention provides for methods of treating food items with the antimicrobial composition comprising silver dihydrogen citrate, citric acid and second antimicrobial agent. Exemplary food items that are treated with the antimicrobial compositions include vegetables and fruits. Exemplary vegetables that may be treated with the antimicrobial compositions include: roots (such as carrots, beets, radishes); tubers (such as potatoes, turnips, sweet potatoes and yams); bulbs (such as onions, scallions); corms (such as garlic); green leafy vegetables (such as spinach, kale, lettuce and cabbage); cruciferous sprouts (such as broccoli and cauliflower); and legumes (such as beans and peanuts). Exemplary fruits that may be treated with the antimicrobial compositions include: squash, melons, apples, peaches, pears, bananas, tomatoes, citrus (such as oranges, grapefruit, tangerines, tangelos, lemons and limes), grapes and olives. The invention also provides for methods of treating a food item by spraying or wiping the antimicrobial composition onto the food item. The invention alternatively provides for dipping the food item into a the antimicrobial composition to achieve an antimicrobial effect.

The invention also provides methods of treating a variety of articles with the antimicrobial silver dihydrogen citrate, citric acid and second antimicrobial agent

composition. For example, the antimicrobial compositions may be sprayed or wiped onto a variety of items including mechanical parts used in food service or food process manufacturing. The antimicrobial compositions may also be used to clean toys and other items handled by children and infants. The antimicrobial compositions may further be used to clean fabric items, such as clothing, wash rags, bedding and other fabric items.

The invention also provides methods of treating water with the antimicrobial compositions. One such method comprises providing an antimicrobial composition having a pre-selected concentration of silver dihydrogen citrate and halogen compound. A suitable, illustrative, concentration of silver dihydrogen citrate for such composition is in the range of about, especially about 1,000 ppm to about 5,000 ppm; and a suitable, illustrative, concentration for halogen compound in the composition is in the range of about 2,000 to about 20,000 ppm. An amount of the antimicrobial composition is then added to the water container to obtain a silver dihydrogen citrate concentration of about 0.1 ppm to about 50 ppm, especially about 0.2 ppm to about 20 ppm, and a halogen compound concentration of about 0.5 ppm to about 50 ppm, especially about 1 to 10 ppm. The water in the water container is then circulated to ensure a thorough mixing of the silver dihydrogen citrate and halogen compound throughout. It is useful to test the silver dihydrogen citrate level, the halogen compound level, or both subsequent to addition of the antimicrobial composition in order to ensure that an antimicrobial amounts of silver dihydrogen citrate and halogen compound remain in the water container. It is known, for example, that chlorine and bromine compounds are exhausted in the presence of organic matter (leaves, branches), biological material (bacteria, algae), and other foreign matter that may be introduced into the water container. Thus, if the water container is cloudy or is otherwise believed to contain organic material or biological infestation, the water should be checked periodically after addition of the antimicrobial composition to ensure that a silver dihydrogen citrate concentration in the above-mentioned ranges is maintained.

The invention provides methods of using antimicrobial compositions comprising silver dihydrogen citrate and halogen compounds. Such antimicrobial compositions are prepared as described above, with silver dihydrogen citrate being present in an amount of about 1,000 ppm to about 5,000 ppm and halogen compound being present in the range of

about 2,000 ppm to about 20,000 ppm. For treatment of water supplies, drinking water, pools and spas, an amount of the above-mentioned composition is added to the water to obtain concentrations in the range of about 0.1 to about 100 ppm of silver dihydrogen citrate, and about 0.2 to about 20 ppm halogen compound. For surface disinfection, treatment of food articles and non-food articles, the compositions may be diluted to prepare a composition of about 0.1 to about 100 ppm silver dihydrogen citrate and about 0.2 to about 20 ppm halogen compound. The diluted solution is then applied to the food article, non-food article or surface to be treated. Application of the solution to the article or surface will give rise to both immediate and long-term antimicrobial effects.

The invention further provides methods of treating solid surfaces, such as those encountered in the food preparation and food services industries. One such method employs an antimicrobial composition having pre-selected concentrations of silver dihydrogen citrate and a quaternary ammonium compound. A suitable concentration of silver dihydrogen citrate for the treatment composition is in the range of about 0.1 ppm to about 100 ppm silver dihydrogen citrate and about 0.2 ppm to about 20 ppm quaternary ammonium compound. The treatment composition is then applied to the solid surface, such as a stainless steel surface. This method provides extended residual protection from microbial infestation. Once the quaternary ammonium compound has dissipated, the silver dihydrogen citrate persists, providing extended antimicrobial benefit to the solid surface, and hence any materials that may come in contact with the surface.

The invention provides another method for treating solid surfaces, which employs an antimicrobial composition comprising silver dihydrogen citrate, citric acid and an oxidizing agent. The antimicrobial composition has a pre-selected concentrations of silver dihydrogen citrate and oxidizing compound. A suitable concentration of silver dihydrogen citrate for the treatment composition is in the range of about 0.1 ppm and 100 ppm silver dihydrogen citrate; and a suitable concentration for oxidizing agent in the treatment composition is in the range of about 0.2 ppm to about 20 ppm oxidizing agent. The antimicrobial composition is applied to solid surface, such as a stainless steel surface or countertop. This method provides extended residual protection from microbial infestation. Once the oxidizing agent has dissipated, a silver dihydrogen citrate residue persists, providing extended antimicrobial

benefit to the solid surface, and hence any materials that may come in contact with the surface.

The invention further provides a method of treating a substrate, such as a food article or a non-food article with an antimicrobial composition comprising silver dihydrogen citrate, citric acid and chlorine or bromine. A suitable concentration of silver dihydrogen citrate for the antimicrobial composition is at least about 0.1, especially at least about 0.5 ppm. A suitable concentration for chlorine or bromine in the treatment composition is at least about 0.2 ppm, particularly in the range of about 0.2 ppm to about 20 ppm. The treatment composition is applied to the substrate, which may be a solid surface, a food article or a non-food article. This method provides synergistic antimicrobial efficacy.

The invention may be more fully appreciated with reference to the following illustrative and non-limiting examples.

Examples

Example 1: Production of Silver Dihydrogen Citrate Stock Solution

Water was introduced into a reverse osmosis unit, passing through a semi-permeable membrane to remove impurities and producing deionized water. Anhydrous 99% pure citric acid was mixed with the water to produce 200 gallons of a 20 % (wt/vol) (796 g citric acid per gallon water) solution. The 200 gallons of 20% citric acid were directed into an ion chamber containing having positive and negative electrodes, each consisting of 200 troy ounces of 999 fine silver. The positive and negative electrodes were spaced at least 2.0 mm apart, allowing the citric acid solution to pass between the two electrodes. An ion generation controller (IGC) power supply including a positive and a negative conductor was attached to the positive and negative electrodes. The IGC applied a current of 5 amps at 17 volts, pulsed every 9 seconds, with a polarity change at 1 minute intervals. Throughout the process, the electrode gap was adjusted in order to maintain the 5 amp-17 volt output. The electric current flow caused an ion current to flow between the positive and negative electrodes, producing free silver ions within the diluted citric acid solution. The silver ions reacted with the citric acid in the citric acid solution to produce the silver dihydrogen citrate solution. The 20% citric acid solution was recirculated through the ion chamber at 50 gallons per minute

for 144 hours until the desired silver ion concentration was obtained. The silver dihydrogen citrate solution was then allowed to sit in order to allow any solids formed during the procedure to precipitate. The resulting product was a silver dihydrogen citrate solution having a silver ion concentration of 2400 ppm. Hereafter, this solution is referred to as a stock solution. The stock solution can be used immediately per the following examples or stored for later use.

It should be understood by those skilled in the art that numerous variations in the size and/or spacing of the electrodes and numerous variations in the peak voltage and numerous variations in the timing sequence of the intermittent voltage polarity can readily be used to obtain the silver dihydrogen citrate for use in the invention.

Example 2: Production of a Composition of Silver Dihydrogen Citrate and a Quaternary Ammonium Compound, an Oxidizing Agent or a Halogen Compound

(a) A scented, colored intermediate composition: In a 1,000 ml Pyrex™ glass flask, there were combined the ingredients set forth in Table 1 in the indicated proportions:

Table 1

Ingredient	Proportion
Pure Pharmaceutical-Grade Water	98.882%
Quaternary Ammonia 80% Concentrate	0.300%
Triton X-100™ surfactant	0.500%
Non-toxic Scent	0.300%
Non-toxic color	0.015%

(b) Scented, non-colored composition: In a 1,000 ml Pyrex™ glass flask, there were combined the ingredients listed in Table 2 in the indicated proportions:

Table 2

Ingredient	Proportion
Pure Pharmaceutical-Grade Water	98.897%
Quaternary Ammonia 80% Concentrate	0.300%
Triton X-100™ surfactant	0.500%
Non-toxic Scent	0.300%

(c) Non-scented, colored composition: In a 1,000 ml Pyrex™ glass flask, there were combined the ingredients listed in Table 3 in the indicated proportions:

Table 3

Ingredient	Proportion
Pure Pharmaceutical-Grade Water	99.182%
Quaternary Ammonia 80% Concentrate	0.300%
Triton X-100™ surfactant	0.500%
Non-toxic color	0.015%

(a) Non-scented, non-colored composition: In a 1,000 ml Pyrex™ glass flask, there were combined the ingredients listed in Table 4 in the indicated proportions:

Table 4

Ingredient	Proportion
Pure Pharmaceutical-Grade Water	99.197%
Quaternary Ammonia 80% Concentrate	0.300%
Triton X-100™ surfactant	0.500%

The resulting intermediate compositions from (a)-(b) above are mixed for about 15 minutes before adding 0.003% of stock silver dihydrogen citrate solution from Example 1. All the ingredients are then mixed for the remainder of a 1 hour time period. The resulting composition (test solutions (a)-(d)) is then used as in Example 3.

Example 3: Antimicrobial Effect of the Composition from Example 3

An *E. coli* test strain (*E. coli* PIPSA, German H.) was grown at 35°C for 24 hours. The cells were harvested by centrifugation for 10 minutes and were washed twice with Butterfield's Phosphate Buffer (BPB pH. 7.2). The cells were then re-suspended in BPB to obtain a cell suspension of approximately 10^8 CFU/ml. (Target inoculum levels were approximately 10^6 CFU/ml in the final test solution).

Three sets of two non-porous glass slides were provided. For each set of test slides, one was treated with the test solution in Example 3 and the other slide was treated with the intermediate test solution from Example 3. Each slide was then inoculated with *E. coli*.

After inoculation, the test slides were then stored for periods of 1 and 24 hours, then plated out at the indicated time period and incubated for a period of 24 hours. A score of Pass indicates no bacterial growth. A score of Fail indicates bacterial growth. The results in Table 5 below demonstrate the residual activity of a combination of silver dihydrogen citrate and quaternary ammonium compound, which properties are not possessed by compositions comprising the quaternary ammonium compound alone.

Table 5

Sampling Interval/Ingredient	1 Hour	24 Hour
Silver Dihydrogen Citrate + Quaternary Ammonium Compound	Pass	Pass
	Pass	Pass
	Pass	Pass
Quaternary Ammonium Compound Only	Pass	Fail
	Pass	Fail
	Pass	Fail

As can be seen from the foregoing examples, the present invention provides significant advantages in the field of antimicrobial methods. The invention provides antimicrobial compositions and methods that possess broad-spectrum antimicrobial properties. In addition, the invention provides compositions and methods that possess synergistic antimicrobial properties. Further, the invention provides antimicrobial compositions that possess extended antimicrobial properties when applied to a substrate.

While the invention has been described with reference to certain exemplary embodiments, the person skilled in the art will recognize that further embodiments may be realized within the scope of the foregoing general description of the invention.

What is claimed is:

1. A composition comprising silver dihydrogen citrate, citric acid and a second antimicrobial agent selected from the group consisting of a quaternary ammonium compound, an oxidizing agent and a halogen compound.

2. The composition of claim 1, wherein the second antimicrobial agent is quaternary ammonia.

3. The composition of claim 1, wherein the second antimicrobial agent is an oxidizer.

4. The composition of claim 3, wherein the oxidizer is selected from hydrogen peroxide, potassium monopersulfate and potassium permanganate.

5. The composition of claim 1, wherein the second antimicrobial agent is a halogen compound.

6. The composition of claim 5, wherein the halogen is chlorine, a hypochlorite, bromine or iodine.

7. The composition of claim 1, further comprising a dispersing agent.

8. The composition of claim 7, wherein the dispersing agent is a detergent.

9. The composition of claim 8, wherein the detergent is sodium dodecyl sulfate or Octoxynol.

10. A method of using the composition of claim 1, comprising applying the composition to a substrate, whereby an antimicrobial effect is achieved.

11. The method of claim 10, wherein the substrate is a solid surface.

12. The method of claim 10, wherein the substrate is a fabric.

13. The method of claim 10, wherein the substrate is an article.

14. The method of claim 10, wherein the antimicrobial effect is a bacteriocidal effect.

15. The method of claim 10, wherein the antimicrobial effect is bacteriostasis.

16. The method of claim 10, wherein the antimicrobial effect is a virucidal effect.

17. The method of claim 10, wherein the antimicrobial effect is a fungicidal effect.

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(54) Title: ANHYDROUS SILVER DIHYDROGEN CITRATE COMPOSITIONS

(57) Abstract: Anhydrous silver dihydrogen citrate compositions comprise silver dihydrogen citrate and citric acid. The anhydrous compositions can be prepared by freeze-drying. The anhydrous compositions can be reconstituted with a suitable diluent to form silver dihydrogen citrate compositions. The anhydrous compositions can be reconstituted and applied to a variety of substrates to impart an antimicrobial effect on the substrates.

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A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : A01N 55/02
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B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. :

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 6,197,814 B1 (Arata) 6 March 2001 (06.03.2001), entire patent.	1-17

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
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INTERNATIONAL SEARCH REPORT

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